



Encapsulation of curcumin in cyclodextrin-metal organic frameworks: Dissociation of loaded CD-MOFs enhances stability of curcumin



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ABSTRACT

Curcumin has been successfully encapsulated in cyclodextrin-metal organic frameworks (CD-MOFs) without altering their crystallinity. The interaction between curcumin and CD-MOFs is strong through hydrogen bond type interaction between the OH group of cyclodextrin of CD-MOFs and the phenolic hydroxyl group of the curcumin. Interestingly, dissolving the curcumin loaded CD-MOFs crystals in water results in formation of a unique complex between curcumin, γ CD and potassium cations. In fact, the initial interaction between curcumin and CD-MOF is crucial for the formation of the latter. This new complex formed in alkaline media at pH 11.5 has maximum absorbance at 520 nm and emittance at 600 nm. Most importantly, the stability of curcumin in this complex was enhanced by at least 3 orders of magnitude compared to free curcumin and curcumin: γ -CD at pH 11.5. These results suggest a promising benign system of CD-MOFs, which can be used to store and stabilize curcumin for food applications.

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1. Introduction

Phytochemicals derived from natural foods including spices, fruits and vegetables have recently drawn great interest because of their health promotion activities. Curcumin (see Fig. 1A), a major component of turmeric, is a yellow pigment extracted from the ground rhizome of curcuma (Sharma, Gescher, & Steward, 2005) commonly used as a spice and food colorant. The last two decades have generated much enthusiasm among researchers to explore the application of curcumin based on its beneficial biological and pharmacological activities. It has been found to have various important biological applications as an anti-inflammatory molecule (Murakam et al., 2008) along with its antioxidant activity and its anti-carcinogenic effect involving inhibition of angiogenesis (Heger, van Golen, Broekgaarden, & Michel, 2014) and its ability to affect major cell signalling pathways (Sing & Aggarwal, 1995). Curcumin is a fluorescent molecule; its photophysical properties are greatly reliant on the polarity of the environment and the pH of the medium (Priyadarsini, 2009). Curcumin has shown itself to be a good candidate for fluorescence probing (El Khoury & Patra, 2013; Patra, El Khoury, Ahmadieh, Darwish, & Tafech, 2012) and

sensing (Mouslmani, Bouhadir, & Patra, 2015; Patra, Aridi, & Bouhadir, 2013) applications. The absorption maximum of curcumin is at 420 nm in the majority of polar solvents, yet in hydrogen bond acceptor and donor solvents, it is shifted to 430–434 nm, excluding methanol where it is around 423–428 nm (Chignell et al., 1994). In acidic media, the absorption maximum of curcumin is at 422 nm, however, at pH >7, the yellow colour of curcumin turns bright red, with the absorption maximum shifting to 463 nm caused by ionization of the phenolic OH group (Bernabé-Pineda, Ramirez-Silva, Romero-Romo, González-Vergara, & Rojas-Hernández, 2004). Curcumin is well known for its instability in neutral and alkaline conditions, undergoing a hydrolytic degradation to feruloyl methane, ferulic acid and vanillin (Tønnesen, Måsson, & Loftsson, 2002). Nevertheless, the exposure of curcumin to alkaline foods or components may be hard to avoid, thus, it should be protected from physical and chemical damage before its industrial use. Several approaches have been tried to improve the delivery of curcumin in its intact hydrophobic form. These include several encapsulation-based systems as polymer nanoparticles (Bisht et al., 2007), phospholipids (Maiti, Mukherjee, Gantait, Saha, & Mukherjee, 2007), polyethylene glycol conjugates (Safavy et al., 2007), and surfactants (Wang, Leung, Kee, & English, 2010). In addition, curcumin can be stabilized by forming metallo-complexes with divalent cations prepared in a water/glycerol system (Zebib, Mouloungui, & Noirot, 2010). However, for food application the encapsulating agent should be non-toxic and edible.

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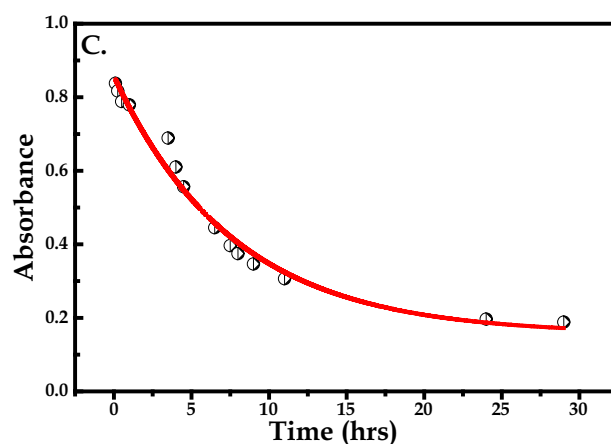
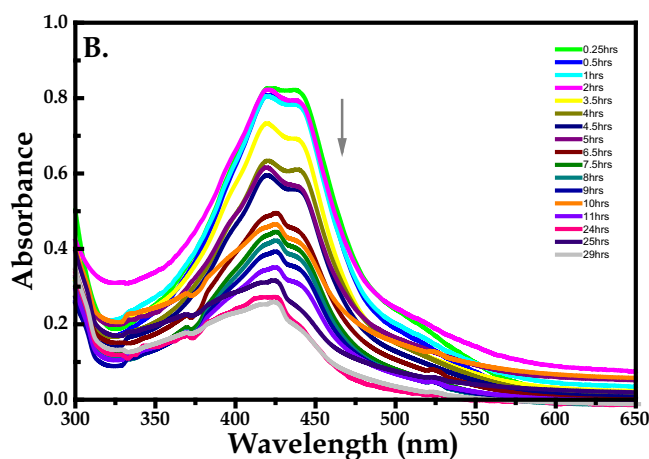
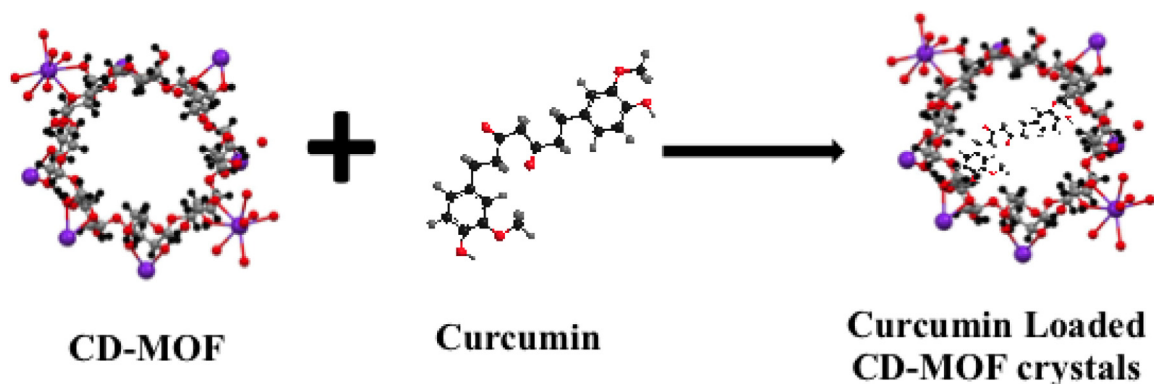


Fig. 1. (A) Interaction between CD-MOF and curcumin; (B) UV-visible absorption spectra of curcumin in the presence of CD-MOFs suspended in methanol with time; (C) Variation of absorbance at 440 nm of curcumin in the supernatant in the presence of CD-MOFs.

Among these possibilities for encapsulation of curcumin for food applications, several cyclodextrin inclusion complexes have been tested (Mangolim et al., 2014).

In the past decade, metal-organic frameworks (MOFs), a novel class of crystalline porous materials, emerged as one of the most investigated areas. Structurally, MOFs are composed of an organic linker and a metal cluster connected mainly through coordination bonds and arranged in an extended 3D network, spatially organized so as to produce cavities of a regular size (Silva, Corma, & García, 2010). Due to their extremely high surface areas, extending beyond 6000 m²/g (Furukawa, Cordova, O’Keeffe, & Yaghi, 2013; Furukawa et al., 2010; Zhou, Long, & Yaghi, 2012) along with a relatively high thermal and mechanical stability, MOFs have demonstrated great potential for a varied array of applications (Deng et al., 2012; Hmadneh et al., 2012; Schlichte, Kratzke, & Kaskel, 2004). Various kinds of MOFs have been reported; one of the important classes is edible MOFs prepared from natural products. Among these edible MOFs, cyclodextrin-MOFs (CD-MOFs), as shown Fig. 1A, is produced by using γ -cyclodextrin (γ -CD) (Choi et al., 2014), a symmetrical cyclic oligosaccharide obtained from starch and composed of eight asymmetric α -1,4-linked Dglucopyranosyl moieties (Smaldone et al., 2010). The building units in CD-MOFs are connected by potassium ions in aqueous media at suitable temperature and pressure, producing a body-centred cubic structure (Smaldone et al., 2010). The storage ability of CD-

MOFs has already been established using two different experimental approaches with rhodamine in an aqueous methanolic solution and 4-phenylazophenol in CH₂Cl₂ (Smaldone et al., 2010). In addition to their selective CO₂ adsorption under low pressure (Wu et al., 2013), CD-MOFs that are synthesized from natural components under benign conditions, hold promise for therapeutic and biological applications. In this study we have investigated the encapsulation of curcumin in CD-MOFs. To the authors’ knowledge this is the first report where encapsulation of curcumin in CD-MOFs has been carried out and the stability of curcumin in such a system is reported. Encapsulation of curcumin in CD-MOFs has been characterized by various methods, revealing that curcumin inhabits the pores of CD-MOFs without affecting their crystallinity. The interactions between curcumin and CD-MOFs were found to be strong through a hydrogen bond type interaction between the OH group of the cyclodextrin moiety of CD-MOF and the phenolic hydroxyl group of curcumin. The absorption and fluorescence spectral study indicated that the pores encapsulating curcumin in CD-MOFs have a similar solvent environment to methanol. Furthermore, the dissociation of loaded curcumin-CD-MOF crystals in water led to the formation of a unique water soluble complex in which curcumin appeared to be chemically stable in alkaline media (pH = 11.5). These interesting findings pave the way to explore CD-MOFs as a promising benign system in which to store and stabilize curcumin for food applications.

2. Materials and methods

2.1. Material

γ -Cyclodextrin (purity >99%, food grade) was donated by Wacker Chemical Corporation (Adrian, MI, US). Potassium hydroxide, as pellets (ACS reagent, purity \geq 85%), was purchased from Columbus Chemical Industries, Inc. (Phoenix, AZ, US). Methanol (purity >99.8%) was purchased from Sigma-Aldrich Corp. (Saint Louis, MO, US). Dichloromethane (purity >99%) containing 50–150 ppm amylene as stabilizer; chloroform containing 100–200 ppm amylenes as stabilizer (purity \geq 99.5%), and hexane (purity \geq 98%) were also purchased from Sigma-Aldrich Corp. (Saint Louis, MO, US). Diethyl ether (purity >99.8%) and water were purchased from Avantor Performance Materials (Center Valley, PA, US). Curcumin was obtained from Sigma-Aldrich and used as received.

2.2. Sample preparation

CD-MOFs were synthesized from food grade components as described by (Smaldone et al., 2010) whereby 1.0 mole-equivalent of the cyclodextrin (1.30 g) was dissolved in 20 mL deionized distilled water with 8.0 mole-equivalents of potassium hydroxide (0.45 g) under magnetic stirring for 6–12 h at 500 rpm. The ratio of metal salt (KOH) to γ -CD was 1:8. The solution was filtered and sealed in a beaker containing 50 mL methanol to allow for vapour diffusion over a period of 7 days at 23 ± 0.1 °C and $50 \pm 2\%$ RH after which a yield of approximately 85% of CD-MOF crystals was obtained (1.5–1.6 g).

The activation process frees the pores from any residual moisture entrapped during synthesis. The crystals were filtered, washed two or three times with 20–30 mL of methanol to remove all extra unlinked potassium ions, and allowed to dry in air for 30 min. The collected crystals were soaked in methanol for 3 days before being filtered and dried under vacuum ($P = 1$ Torr) for 10 h at 25 °C followed by 12 h at 45 °C. This process ensured complete evacuation of the solvents from the pores. Consequently, the crystals were either used immediately or stored over Drierite[®] desiccant.

To study the inclusion of curcumin, 10 mg of CD MOF crystals were dispersed in 3 ml of methanol. Curcumin dissolved in methanol was added to obtain a final concentration 25 μ M. For the spectroscopic measurements the crystal were sonicated to achieve a good dispersion in methanol. However, for the dissolved fraction, the crystals were washed thoroughly with methanol until the supernatant was clear and 3 ml of water were added to dissolve the loaded CD MOF crystals.

2.3. Spectroscopic measurement

UV–visible absorption spectra were recorded using a SCOV-570 UV–vis–NIR spectrophotometer. Steady state fluorescence measurements were performed by a Jobin–Yvon–Horiba fluorimeter, emission and excitation slits were both set at 5 nm, equipped with a 100 W Xenon lamp and an R-928 detector operating at 950 V. FTIR spectra were recorded on an FT-IR-Raman spectrometer Thermo-Nicolet. The spectra were collected in the 4000–650 cm^{-1} range.

2.4. Surface area and pore volume measurements

The surface area of the activated CD-MOF crystals was determined using an autosorb iQ-Micropore-XR (Quantachrome Instruments, Boynton Beach, FL, USA) gas analyzer using nitrogen gas. The Brunauer–Emmett–Teller (BET) and Langmuir methods were

used to determine the surface area with P/P_0 varying from 10–5 to 0.99 P/P_0 . Micropore volumes were calculated at a radius of 2 nm and relative pressure 0.5 P/P_0 , whereas total pore volumes were obtained at the relative pressure $P/P_0 = 0.99$. Samples were run in triplicate.

2.5. X-ray diffraction (XRD)

XRD data was collected for the CD-MOF crystals using a Bruker D8 advance X-ray diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) at 40 kV, 40 mA (1600 W) using Cu $K\alpha$ radiation ($\lambda = 1.5418$ Å), with a 1.2 mm primary beam slit and 2.0 mm detector slit. The X-ray scans were carried out for 2θ between 4 and 40 degrees at 0.02° increments. Data were collected in triplicate.

3. Results and discussion

3.1. Inclusion of curcumin into CD-MOF pores

Given the stability, high surface area and large-pore-aperture characteristics of CD-MOF, in addition to its biocompatibility we decided to study the inclusion of the potent anti-inflammatory molecule curcumin into CD-MOF. Activated crystals of CD-MOF were immersed in methanol, and then curcumin prepared in methanol was added to the soaked CD-MOF crystals. The amount of curcumin in the supernatant was measured through ultraviolet–visible (UV–vis) spectrophotometry. The spectra obtained over a period of 30 h are depicted in Fig. 1B and the characteristic absorbance at 440 nm was examined as plotted in Fig. 1C. The same experiment was carried out for curcumin prepared in methanol in the absence of CD-MOF as a control. In the presence of CD-MOF a continuous decrease in absorbance at 440 nm of curcumin in the supernatant was observed, while for the control (in the absence of CD-MOF) the absorbance remained almost unchanged except that after 7 h a minor decrease was observed. Since reduction in absorbance at the absorption maximum is often related to degradation of curcumin in solution (Wang et al., 1997), the control experiment reconfirms that there is no degradation of curcumin in methanol under our experimental conditions, which is consistent with a previous report (Priyadarsini, 2014). Thus, decrease in absorbance of the supernatant solution in the presence of CD-MOF clearly confirms a reduction in the amount of curcumin in the supernatant due to encapsulation into the CD-MOFs (see Fig. 1C). The encapsulation rate of curcumin into CD-MOFs was estimated by applying first order kinetics. The half-life of encapsulation was determined as 5.41 h, indicating encapsulation into the pores to be a relatively slow process under our experimental conditions. After the encapsulation procedure was completed, the CD-MOF (loaded with curcumin) crystals were washed several times in methanol to remove free and weakly adsorbed curcumin on the surface of the CD-MOFs. The final curcumin loaded CD-MOFs crystals were dark pink. These crystals were used for further characterization to understand the interaction between curcumin and CD-MOF.

3.2. Characterization of the loaded CD MOF crystals

The dark pink coloured crystals were characterized by their IR, powder X-ray diffraction (PXRD) and BET analysis. For characterization, the amount of CD-MOFs used was 30 mg where the concentration of curcumin used for encapsulation was 100 μ M. The FT-IR spectrum (Fig. 2A) showed a large shift in the transmittance of the loaded CD-MOFs compared to that of the free CD-MOFs with the latter transmitting at 3884 cm^{-1} corresponding to the OH stretching of the cyclodextran moiety. In contrast the curcumin-loaded

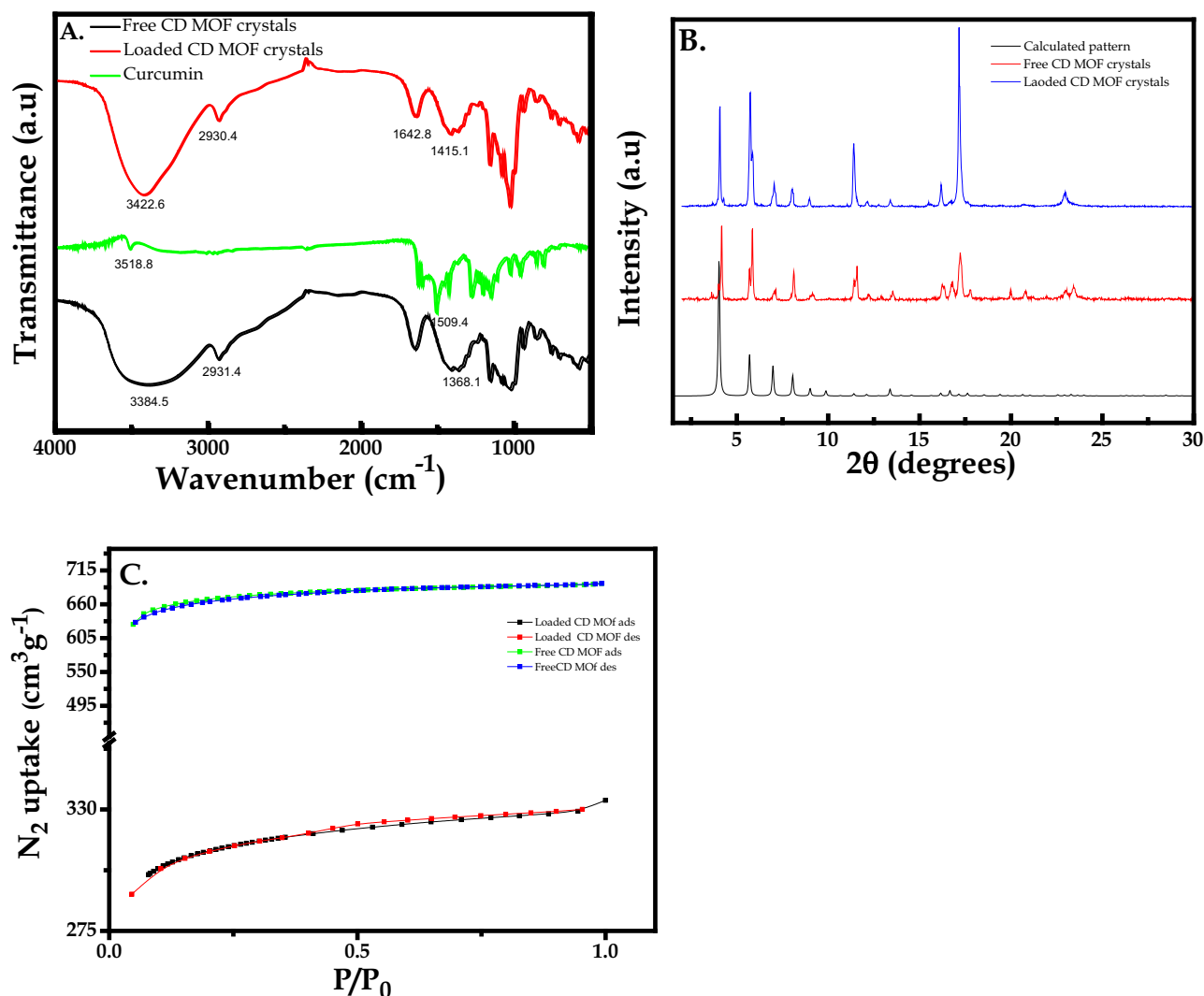


Fig. 2. (A) FT-IR spectra of curcumin, free CD MOF and the curcumin loaded CD-MOFs; (B) Powder X-ray diffraction patterns for free CD-MOFs crystals washed by methanol (in black) and loaded CD-MOFs crystals with curcumin (in red) compared to the calculated pattern of CD-MOFs (blue); (C) N_2 adsorption isotherms, for activated samples of free CD-MOFs and curcumin loaded CD-MOFs. Filled and open symbols represent adsorption and desorption branches, respectively. Connecting traces are for guidance only. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CD-MOFs transmitted at 3422 cm^{-1} , indicating a possible interaction between the OH group of CD-MOFs and curcumin. A prominent peak at 3518 cm^{-1} for the hydroxyl (phenolic) vibration of curcumin was not detected in the FT-IR spectra of curcumin loaded CD-MOF suggesting a hydrogen type of bond between the OH group of the cyclodextrin moiety of CD-MOF and the phenolic hydroxyl group of curcumin. Another major shift was observed where free CD-MOFs transmitted at 1368 cm^{-1} and the loaded sample at 1415 cm^{-1} . Incidentally, these peak positions occurred in the same region as marked absorption by the functional groups of curcumin was demonstrated. A peak at 1600 cm^{-1} for the benzene ring skeleton stretching of curcumin was in the same region as free CD-MOFs. Similarly, 1280 cm^{-1} for Ar–O stretching and 1500 cm^{-1} for C=O and C=C vibrations of curcumin overlapped with free CD-MOFs. Therefore, little can be concluded about the interaction in this region except involvement of the –OH and phenolic group, which could be attributed to an H-bond type of interaction. Interestingly, the broadness in the peak of the free CD-MOFs due to the intra-molecular hydrogen bonding persisted in the loaded CD-MOF, indicating that curcumin does not disturb the structure of the CD-MOFs. The PXRD patterns as depicted in

Fig. 2B further clearly show that curcumin does not disrupt the crystallinity of the CD-MOFs, showing only a slight shift in the positions of the peaks. The porosity of the activated CD-MOF was verified by measuring the N_2 gas adsorption of the sample as shown in Fig. 2C. The typical isotherm exhibited steep N_2 uptake in the low-pressure areas for ($P/P_0 < 0.05$), hence validating the micro porosities of these materials, and the BET surface area for CD-MOF was estimated to be $1030\text{ m}^2\text{ g}^{-1}$. Similarly, the BET surface area of the loaded CD-MOF crystals was measured and found to be $800\text{ m}^2\text{ g}^{-1}$. This decrease in the surface area is a further proof of the interaction taking place between curcumin and the CD-MOFs crystals as curcumin is occupying some of the empty pores of the CD-MOFs.

3.3. Spectroscopic study

After several washings with methanol to remove free and weakly adsorbed curcumin on the surface of the CD-MOFs, the absorption spectrum of the curcumin loaded CD-MOFs crystals dispersed in methanol was measured. As shown in Fig. 3A, the crystals absorbed at 425 which is the characteristic curcumin peak in

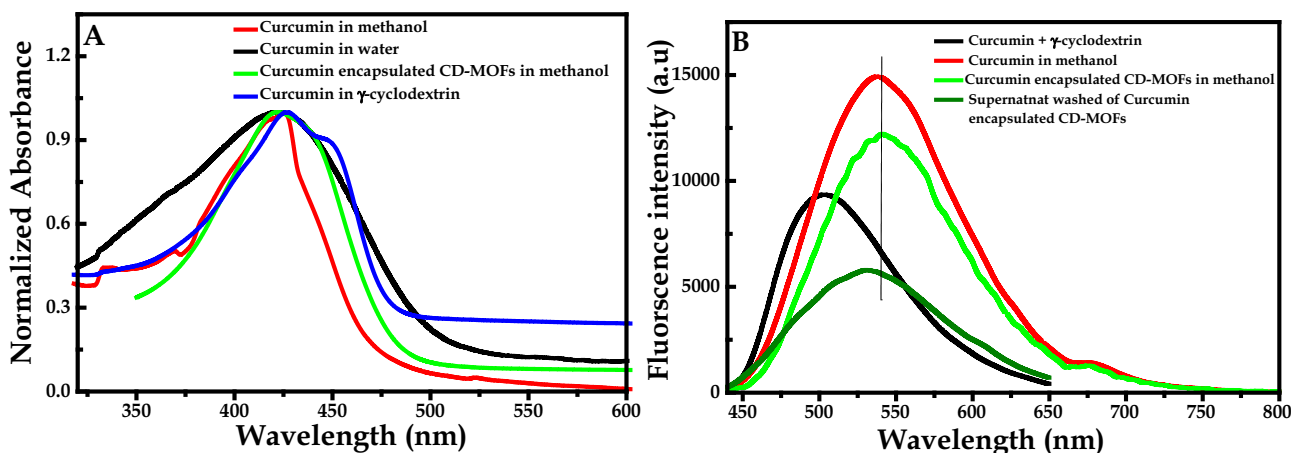


Fig. 3. (A) UV-visible absorption spectra of the free curcumin in water (in black), methanol (in red), in γ -cyclodextrin (in blue) and curcumin encapsulated CD-MOFs crystals in methanol (in green); (B) Fluorescence emission spectra of free curcumin in methanol (red), in curcumin encapsulated CD-MOFs crystals (in green) and that of curcumin in methanol (in black), both excited at 425 nm. Curcumin: γ -CD = 1:10. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

methanol, whereas free CD-MOFs crystals dispersed in methanol showed no absorption around 425 nm. The absorption of CD-MOFs in the visible region is a strong indication for the presence of curcumin within the CD-MOFs crystals, which is directly reconfirmed by the colour change of CD-MOFs after encapsulation of curcumin. The fluorescence spectrum of these dispersed crystals was investigated. The fluorescence emission spectrum, at 425 nm excitation wavelength, (Fig. 3B) obtained for the curcumin loaded CD-MOFs crystals dispersed in methanol is identical to that of curcumin in methanol, thus, the interaction between curcumin and the CD-MOFs crystals preserves the photo-physical properties of curcumin. Curcumin has been shown to be a good probe molecule to sense the local environment of heterogeneous systems (Gogoi & Sen Sarma, 2015; Patra & Sleem, 2013). The fluorescence spectra for curcumin encapsulated CD-MOFs and the supernatant washed off curcumin-loaded CD-MOFs were found to be similar and showed a negligible blue shift compared to curcumin in methanol. In contrast, the fluorescence spectrum of curcumin in γ -cyclodextrin was markedly blue shifted, \sim 40 nm, indicating that the local microenvironment of curcumin inside the γ -cyclodextrin was more hydrophobic and less polar compared to that inside the CD-MOFs. This confirms that the interaction between curcumin and γ -cyclodextrin is different to that between curcumin and CD-MOFs. This could be possible owing to the presence of potassium ions in CD-MOFs, which could facilitate the interaction and/or coordination of curcumin with CD-MOFs. It also establishes that the local microenvironment inside the pores of CD-MOFs is similar to that of methanol.

3.4. Dissociation of curcumin-loaded CD-MOF crystals

Two approaches for the destruction of the CD-MOF structure were followed. The first was dissolving the loaded CD-MOF crystals in water and the second one was treating them with an acidic solution in methanol. It is known that dissolving CD-MOF crystals in water results in dissociation of the framework, hence releasing γ -cyclodextrin and KOH into the solution and thereby creating a basic medium. Interestingly, destroying the curcumin loaded CD-MOFs in water changed the colour of the solution. A pink colour was observed upon dissolving the loaded MOF crystals in water instead of the dark pink colour observed in methanol, and the absorption spectrum showed a maximum at 520 nm (Fig. 4A). In most of the polar solvents, the absorption maximum of curcumin

is at \sim 420 nm, and it is shifted in hydrogen bond acceptor and donor solvents to \sim 430, except in methanol where it is around 425 nm (Priyadarsini, 2014). A shift in the absorption maximum of curcumin is reported in basic medium where curcumin absorbs at 463 nm (Priyadarsini, 2014) with a bright red colour; this shift is caused by the ionization of the phenolic OH group. Shen and Ji (2007) theoretically predicted an absorption maximum at 531 nm for curcumin caused by de-protonation of the phenolic OH group. Moreover, a study performed by (Zsila, Bikádi, & Simonyi, 2003) for curcumin in KOH-ethanolic solution reported an absorption maximum at 535 nm, however, free curcumin molecules are quite unstable at above pH 7 in pure aqueous solution, thereby limiting further investigations of curcumin properties in basic medium. In the current study, when the absorption spectrum of the dissociated curcumin loaded CD-MOF in water showed a broad peak at \sim 520 nm, the pH of the solution was measured and found to be 11.5.

In order to identify the absorption peak and predict the nature of the complex existing at pH 11.5 between curcumin and γ -cyclodextrin in the presence of K^+ ions in water, different control experiments were carried out. In the first case (Fig. 4B), the influence of different molar ratios of curcumin to γ -cyclodextrin on absorption spectrum of curcumin was examined. A solution of curcumin with concentration 25 μ M in water was prepared and to this solution γ -cyclodextrin solution was added in portions to produce various curcumin to γ -cyclodextrin molar ratios. The absorption maximum of curcumin was not affected by a different molar ratio of γ -cyclodextrin, but as the molar ratio of γ -cyclodextrin (curcumin: γ -cyclodextrin = 1:0–1:10) increased the absorption spectrum became narrower. Narrowing of absorption spectra of curcumin in micellar medium has also been observed (Ghosh & Mondal, 2012) and can be an indication that at higher molar ratio most of the curcumin molecules are completely buried inside the hydrophobic pocket of γ -cyclodextrin. It has also been reported that one curcumin molecule can bind to two cyclodextrin units (Mohan, Sreelakshmi, Muraleedharan, & Joseph, 2012), thus the need for a higher molar ratio with γ -cyclodextrin for complete encapsulation would be expected, though spectra for 1:7 and 1:10 were found to be identical due to saturation. This excludes the possibility that the band at \sim 520 nm observed for curcumin with the dissociated CD-MOFs framework is due to curcumin- γ -cyclodextrin complex formation. The second possibility could be due to complexation between curcumin and K^+ ion. Absorption

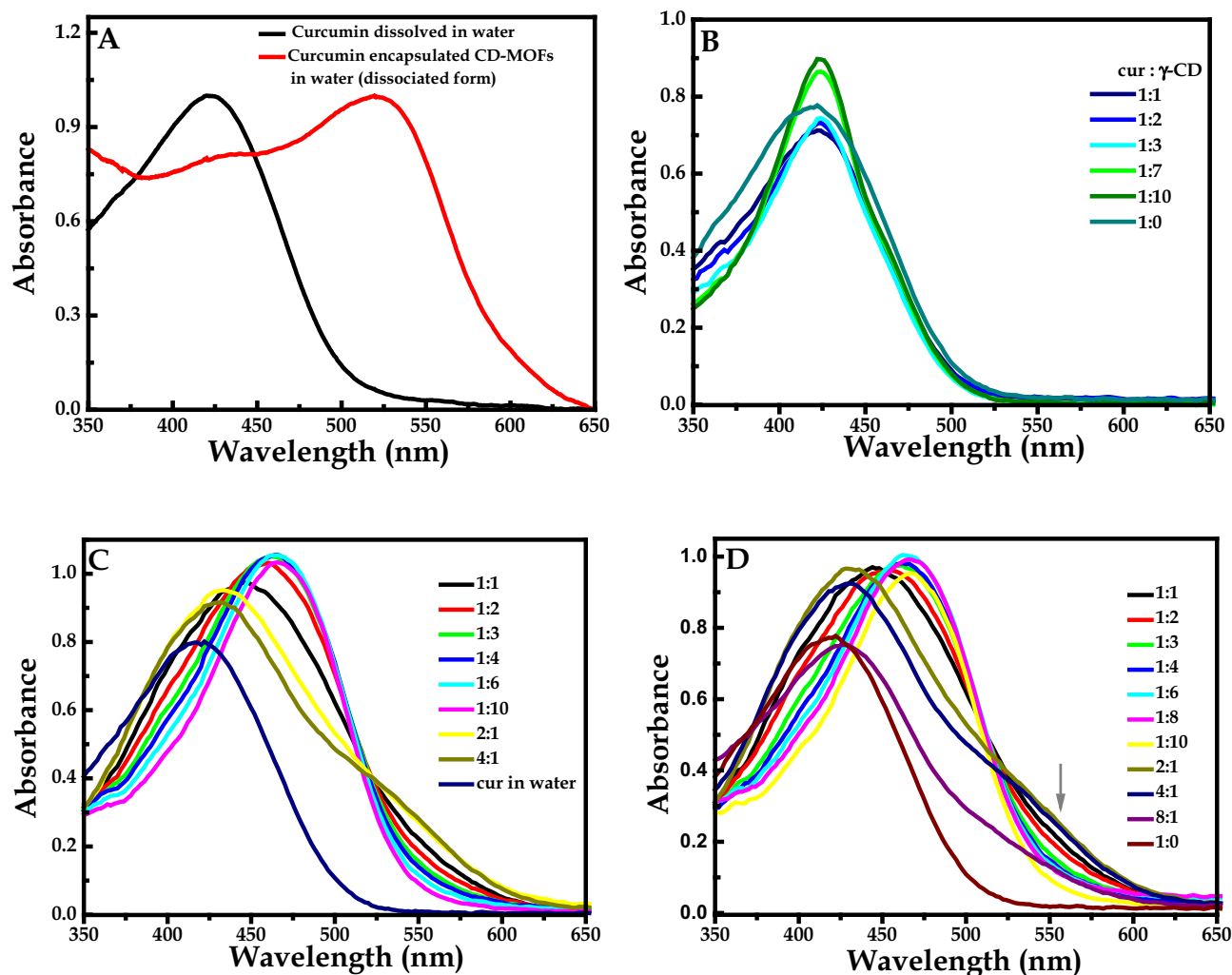


Fig. 4. (A) UV-vis absorption spectrum of the dissolved loaded CD-MOF crystals (red) with curcumin in water and that of curcumin alone in water (black); (B) UV-vis absorption spectrum of curcumin (25 mM) in the presence of different concentrations of γ -cyclodextrin in water; (C) UV-vis absorption spectrum of curcumin (25 mM) in different concentrations of KOH in water; (D). UV-vis absorption spectrum of curcumin (25 mM) in different concentration of (γ -CD:KOH) complex in water. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

spectra of curcumin and KOH at various molar ratios are shown in Fig. 4C. As illustrated in the spectra, a 1:1 M ratio of curcumin:KOH red shifted the absorption spectra to ~ 450 nm with a new minor peak (band) at ~ 550 nm. A further increase in the molar ratio of curcumin:KOH from 1:1 to 1:10 shifted the spectra more towards the red wavelength region, however, the band at ~ 550 nm decreased and completely disappeared at 1:10 of curcumin:KOH. The red shift is expected as in completely deprotonated form curcumin has an absorption maximum at ~ 463 nm (Priyadarsini, 2014). Interestingly, when the molar ratio of curcumin:KOH was increased to 2:1 and 4:1 the main peak at ~ 450 nm (for 1:1 M ratio) showed a blue shift with an enhancement of the band at ~ 550 nm, suggesting this band could be due to one potassium ion bound to multiple curcumin molecules. To test whether γ -cyclodextrin had any role in this complex, a solution of γ -cyclodextrin to KOH (1:1) was prepared and added in portions to a 25 μ M curcumin solution. Strikingly, the absorption spectra (Fig. 4D) showed a shoulder at ~ 530 nm instead of ~ 550 nm (Fig. 4C) for the solutions of 2:1 and 4:1 curcumin to γ -CD-KOH solution (Fig. 4D). At a higher concentration of curcumin in the 8:1 solution, no shoulder appeared. As well as going from 1:1 to 1:10, no shoulder was found, but a shift in the main absorption peak to ~ 465 nm occurred as a result of the deprotonated form

of curcumin. This spectral study proposes that the band observed at ~ 520 nm in the dissociated loaded CD-MOF was due to the formation of an adduct between curcumin, potassium ion and γ -cyclodextrin.

To study the importance of encapsulation of curcumin in the extended porous structure of CD-MOFs in the formation of the adduct, we investigated the association of curcumin with dissociated CD-MOF was investigated and compared with that of dissociated curcumin-loaded CD-MOFs. As depicted in Fig. S1A (see Supporting information), when curcumin was added to dissolved CD-MOFs in water, a coloured solution was obtained; the absorption spectrum gave a maximum at ~ 450 nm and a weak intensity shoulder at 520 nm. Due to the weak intensity of the 520 nm shoulder which is characteristic of the adduct formation (Fig. 4A), it can be concluded that the formation of this complex via addition of curcumin to the dissolved CD-MOF was not favourable. This result clearly demonstrates the importance of the encapsulation of curcumin within the pores of the CD-MOF crystals prior to the formation of the water soluble adduct. To appreciate the role of water molecules during the complex formation, the curcumin-loaded CD-MOF crystals were dissolved in methanol solution by adding hydrochloric acid to the MOF crystals dispersed in methanol. It is well established that treating MOFs structures with acid

results in the dissociation and destruction of the framework (Zhou et al., 2012). As shown in Fig. 5A, after treating the loaded CD-MOFs crystals with acid the absorption spectrum was identical to that of curcumin observed in methanol, although it was slightly broadened like that of curcumin in water. Thus, water plays a significant role during the complex formation via dissociation of curcumin-loaded CD-MOF in water.

In order to study the emission properties of the adduct formed via dissociation of curcumin loaded CD-MOFs, fluorescence spectra of the complex were recorded at $\lambda_{\text{ex}} = 425$ nm and 520 nm and compared to the emission spectra of free curcumin. As shown in Fig. 5B, curcumin at pH 11.5 excited at 425 nm gave an emission spectrum with a maximum at ~ 540 nm. On the other hand, when excited at 520 nm, as expected no emission was found as curcumin does not absorb at this wavelength. Similarly, the dissolved curcumin-loaded CD-MOF crystals were excited at 425 nm and 520 nm. When excited at 425 nm, the dissolved curcumin loaded CD-MOF crystals (the obtained adduct) gave a moderately intense fluorescence emission spectra with an emission maximum at ~ 600 nm. This emission shows a marked ~ 50 nm red shift from curcumin alone, which could have two explanations, either a change in the local environment of curcumin, or a result of a

new emissive species. Interestingly, when excited at 520 nm the adduct showed an emission spectrum with a marked enhancement in fluorescence intensity and an emission maximum at ~ 600 nm. This result confirms that at both excitation 425 nm and 520 nm the same species is emitting; this species is different from the curcumin alone, thus demonstrating a cooperative complex formation between curcumin, potassium ion and γ -cyclodextrin.

To understand the pH dependent stability of the dissociated curcumin-loaded CD-MOFs crystals, the absorption and fluorescence spectra were recorded at different pH values using buffer solutions. Fig. 51B (see Supporting information) shows absorption spectra of dissolved curcumin loaded CD-MOFs at different pH values. Interestingly, the absorption at 520 nm appeared only at pH 11.5 and at lower or higher pH the absorption spectra were similar to that of curcumin. For instance, absorption of curcumin loaded CD-MOF crystals gave a blue shift at pH 13–470 nm, and was further shifted at pH 4, 5, 7 and 10–430 nm. In Fig. 5C, the effect of pH on the fluorescence spectrum was presented. When excited at 425 nm (see Fig. S1C, Supporting information), the curcumin loaded CD-MOF crystals at pH 4, 5, 7 and 8 emitted in the region 450–600 nm, with a maximum at ~ 540 nm, indicating that the emission was solely due to curcumin, whereas at pH 11.5 the

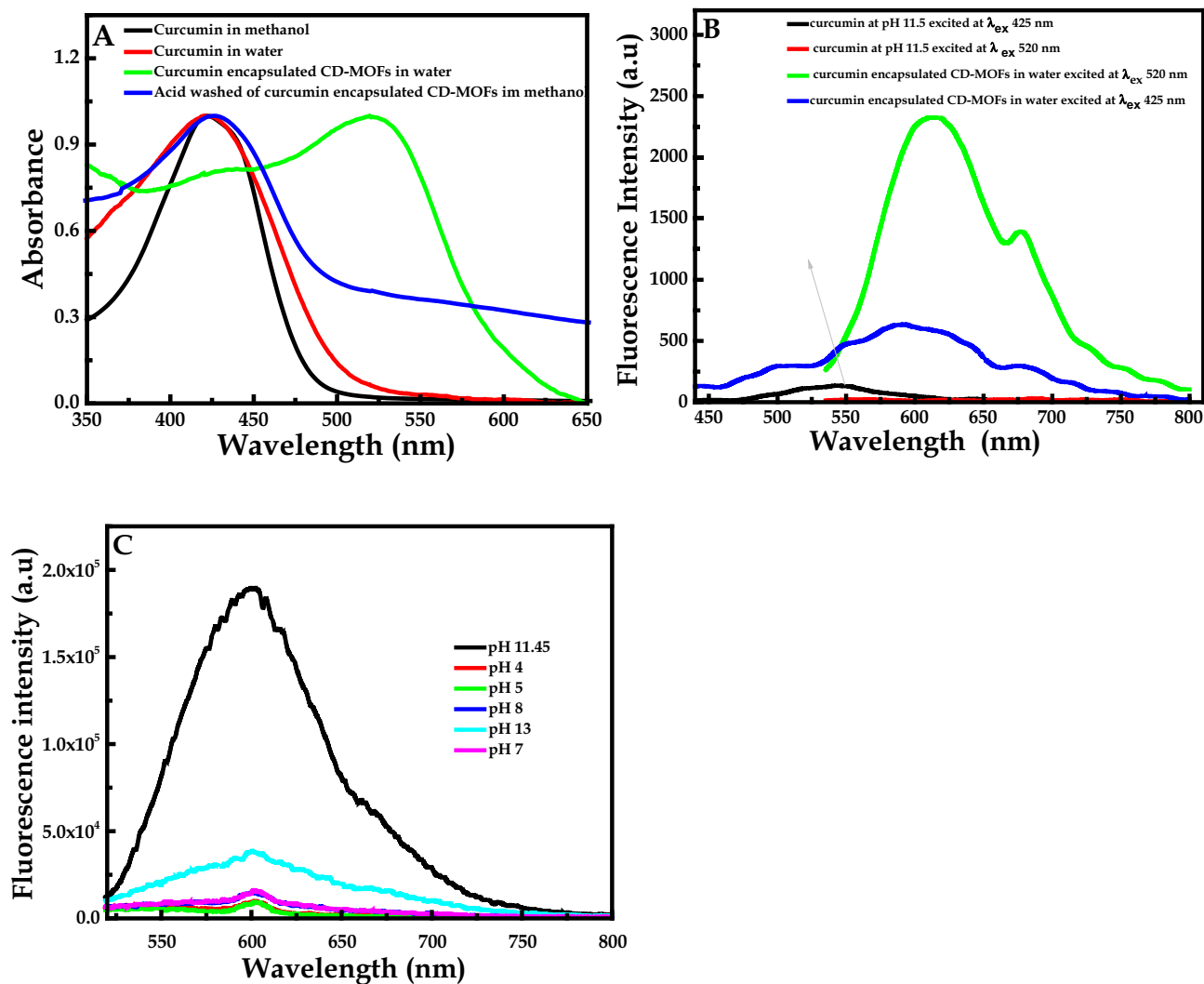


Fig. 5. (A) UV-vis absorption spectra of the acid washed loaded CD-MOFs crystals (in blue), curcumin in methanol (in black), curcumin in water (in red), and dissolved loaded CD-MOF crystals in water (in green); (B) Fluorescence spectrum of the dissolved loaded CD-MOFs crystals in water excited at 425 nm (blue) and 520 nm (green). For comparison, fluorescence spectrum of curcumin at pH 11.5 excited at both 425 nm (black, inset) and 520 nm (red); (C) Fluorescence spectrum of the dissolved loaded CD-MOFs crystals in water in different pH excited at 520 nm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

emission was in the 500–700 nm region with a maximum at ~600 nm. At pH 13 a similar spectrum with lower intensity was obtained, suggesting that at this pH a small amount of the potassium-curcumin was still present. As expected when excited at 520 nm (Fig. 5C), there was no fluorescence observed at pH 4, 5, 7 and 8, whereas a low fluorescence was obtained at pH 13 and a remarkably high fluorescence intensity with an emission maximum at ~600 nm was found for pH 11.5, which further demonstrated that the adduct had a strong absorption and emission properties that were different from that of curcumin. Curcumin has 3 pKa values ranging from 7.75 to 10 (Bernabé-Pineda et al., 2004). Therefore, the complexation occurred with a completely deprotonated form of curcumin and as soon as the pH of the medium was lowered, the fluorescent complex collapses. Nevertheless, increasing the pH further to more alkaline medium, ~pH 13, the complex was not stable. This finding indicates that the potassium ion of CD-MOFs was associated with at least one phenolate ion and another enolic ion of curcumin.

3.5. Stabilization of curcumin in dissociated CD-MOF

In the first part of this study, the physical interaction between curcumin and the CD crystals demonstrated by the IR spectrum, UV-visible and fluorescence spectra was studied and it was demonstrated that the incorporation of curcumin in the cavities

of CD-MOF is crucial for the formation of the adduct upon dissolving the loaded CD-MOF. The second part of this study investigated the stability of curcumin in the CD-MOF structure. Several studies have reported the interaction of curcumin with the family of cyclodextrin, such as α , β , and γ -CD (Mohan et al., 2012; Szente, Mikuni, Hashimoto, & Szejtli, 1998; Tang, Ma, Wang, & Zhang, 2002). Curcumin is relatively stable in organic solvents like methanol but it is quite unstable in aqueous alkaline medium. Similarly, stabilization of curcumin with cyclodextrin has been reported in alkaline medium at pH 8 (Tønnesen et al., 2002). There are succeeded in stabilizing curcumin at high pH conditions. The effect of interaction of curcumin with CD-MOF crystals on the degradation of curcumin has been investigated in water since curcumin is relatively stable in organic solvents. It is noteworthy that dissociation of the loaded crystals in water leads to the formation of a water soluble complex that involves association between curcumin, potassium cations and CD. In the current study, the UV-vis absorption spectra of the dissolved curcumin loaded MOF crystals were recorded for 8 h in water under alkaline condition as shown in Fig. 6. The degradation rate was fitted to first order kinetics as shown in Fig. 6D. Free curcumin showed a high level of degradation after 2 h that continued for an 8 h period (See Fig. 6D). The results illustrated that curcumin undergoes rapid degradation at pH 11.5 with a half-life of 1.45 h. Recent work has established that curcumin exists in solution in the keto-enol tautomeric form

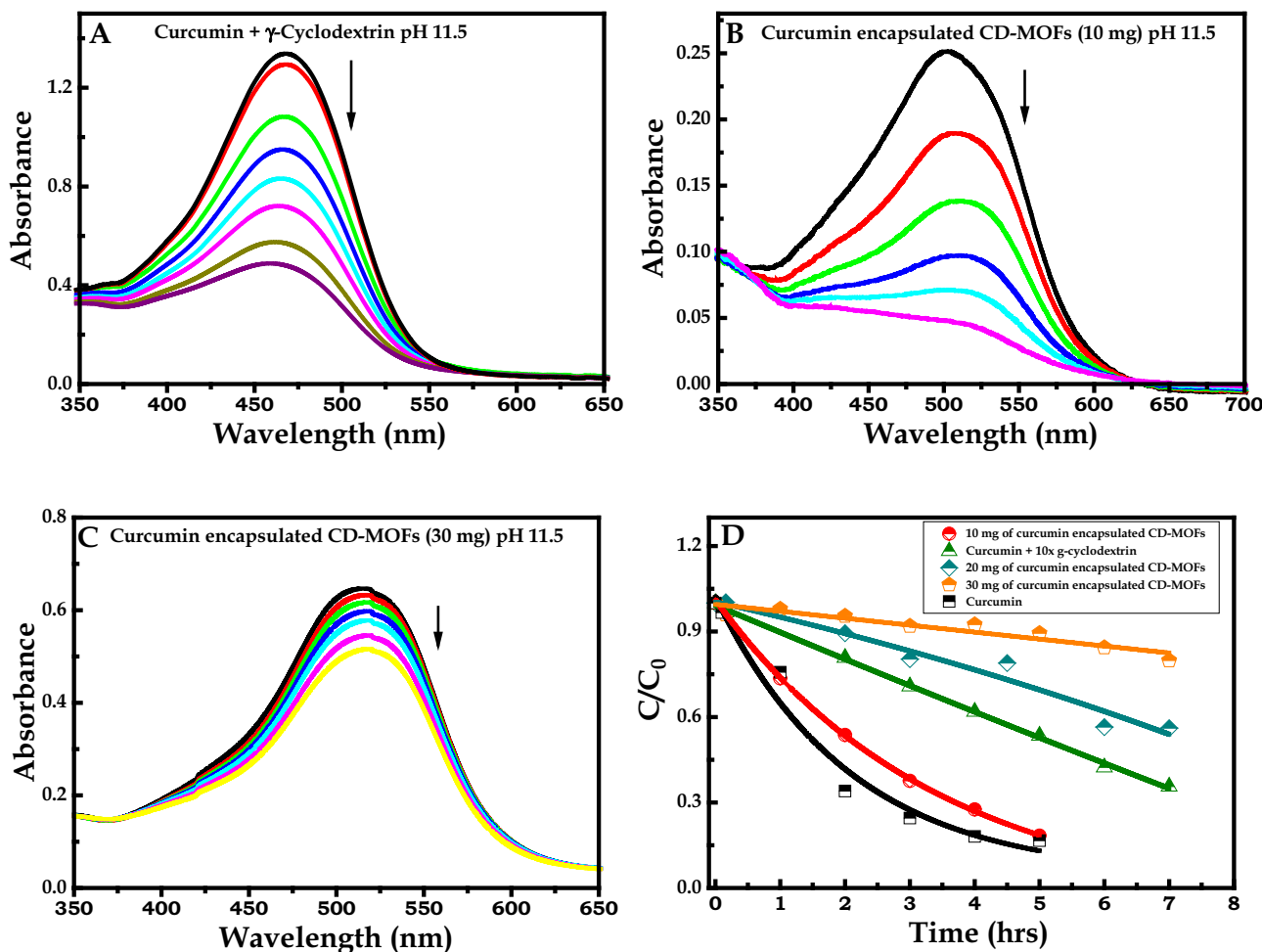


Fig. 6. UV-visible absorption spectra of curcumin degradation in (A) γ -cyclodextrin (molar ratio 1:10 for curcumin: γ -cyclodextrin), (B) 10 mg CD-MOFs and (C) 20 mg CD-MOFs; (D) Degradation of curcumin in pH 11.5. Free curcumin at pH 11.5 (red), curcumin with 10 mg of CD-MOF crystals (green), curcumin with 20 mg of CD-MOF (black), curcumin with 30 mg of CD-MOF crystals (blue) and curcumin with 10 times g-cyclodextran at pH 11.5 (cyan). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Priyadarsini, 2014). Under alkaline conditions, the enol group of curcumin becomes de-protonated and it is likely to undergo degradation by a retro-aldol condensation reaction catalyzed by the hydroxide anion. In the past, curcumin has been encapsulated in p-sulfonatocalix[4]arene, but in alkaline conditions (pH 9.2) degradation of curcumin has been unaffected in p-sulfonatocalix[4]arene (Mareeswaranm et al., 2014). On the other hand, stability of curcumin in γ -cyclodextrin has been reported (Harada et al., 2011; Tønnesen et al., 2002). For comparison, in the present study, the stability of curcumin was investigated at 10 times the concentration of γ -cyclodextrin (so that all the curcumin molecules present in solution were bound to γ -cyclodextrin) as shown in Fig. 6A. Degradation of curcumin in the presence of γ -cyclodextrin continued with a half-life of 56 h, which is ~ 39 times more stable than curcumin alone. Harada et al. have reported a half-life of ~ 0.09 h for free curcumin and 4.46 h for γ -cyclodextrin in PBS buffer at pH 7.4 (Harada et al., 2011), thus, the current results are an improvement on those reported in the literature. Since pH of the buffer solution and molar ratio of cyclodextrin:curcumin could influence stability of curcumin, the higher stability of curcumin in γ -cyclodextrin must be due to the higher molar ratio of cyclodextrin:curcumin (10:1) in our case compared to the 1:1 M ratio by Harada et al. (2011). When 10 mg of the curcumin-encapsulated CD-MOFs crystals were used, as can be seen from Fig. 6B, the measurement showed no appreciable difference to that of free curcumin at pH 11.5 in water, having a half-life of 2.36 h (a marginal increase). However, as the amount of CD-MOFs increased to 20 mg, the stability of curcumin increased to a half-life value of 10.22 h, which is still lower than that of curcumin in 10 times the concentration of γ -cyclodextrin. A further increase of CD-MOFs to 30 mg increased the stability of curcumin (Fig. 6C) to a half-life value of above one million hours at pH 11.5. Thus, in the presence of a sufficient amount of CD-MOFs, the stability of curcumin was substantially enhanced due to incorporation within the pores of the crystals.

4. Conclusion

Curcumin was successfully encapsulated in CD-MOFs. The encapsulation kinetics were relatively slow with a half-life of 5.41 h under the current experimental conditions. The results established that curcumin sits in the pores of CD-MOFs through a hydrogen bond type interaction between the OH group of the cyclodextrin moiety of CD-MOFs and the phenolic hydroxyl group of curcumin. Moreover, the presence of curcumin does not disturb the crystallinity of CD-MOFs. The solvent polarity of the pores inside the CD-MOFs was found to be methanolic. Dissociation of the curcumin-loaded CD-MOF in water did not separate curcumin from the dissolved framework but resulted in formation of an adduct in which the interaction was found to be unique in nature through complexation of potassium ions with curcumin and CD. The formation of this new complex, which absorbed at 520 nm and emitted at 600 nm, depends strongly on the incorporation of curcumin within the pores of CD-MOFs. The dissociated framework incorporating curcumin molecules showed an important enhancement in the chemical stability of curcumin in water in alkaline conditions. In conclusion, CD-MOFs could be a promising benign system in which to store and stabilize curcumin for food applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2016.06.013>.

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